NOTE

Pathology

A Case of Canine Vasotropic and Vasoinvasive Nonepitheliotropic Lymphoma with Unusual Tumor Cells and Extensive Dermal Necrosis

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Abstract. A 14-year-old, spayed female Shih-tzu dog presented with masses in the dorsal aspect of cervical region and digit of the right anterior limb. Extensive necrosis was seen in the dermal tissue overlying the tumor, and diffuse round cell proliferation and infiltration were seen histologically from the superficial dermis to the deep dermis. Two types of proliferating cells were present: lymphoblast-like cells with round-oval, vesicular nuclei and moderate-large nucleoli; and smaller cells with characteristic irregularly shaped nuclei. Electron microscopy of these smaller cells showed cerebriform, pleomorphic nuclei with a chromatin pattern characteristic of lymphoid cells, as seen in lymphoblast-like tumor cells. Immunohistochemically, both types of tumor cells were positive for CD3. Most vessel walls had been invaded by tumor cells, resulting in extensive dermal necrosis and hemorrhage. Based on these histopathological findings, the tumor was diagnosed as vasotropic and vasoinvasive nonepitheliotropic lymphoma, characterized by a notable presence of unusual tumor cells with irregularly shaped nuclei and extensive dermal necrosis.

Key Words: canine, vasotropic and vasoinvasive nonepitheliotropic lymphoma.

Canine cutaneous lymphomas represent about 3–8% of all canine lymphomas, and can be histologically and clinically divided into epitheliotropic and nonepitheliotropic lymphomas [3, 9]. In nonepitheliotropic lymphoma, solitary or multiple, discrete dermal and subcutaneous nodules may develop, with the majority of these tumors displaying a T-cell origin [3]. Vasotropic and vasoinvasive nonepitheliotropic lymphoma is subtyped as nonepitheliotropic lymphoma characterized by vasoinvasive, angiocentric proliferations of neoplastic lymphocytes [3, 11], with lesions involving the face, eyelids, mucocutaneous junctions and trunk [3]. Early lesions of vasotropic and vasoinvasive nonepitheliotropic lymphomas are pleocellular and may display features of a granulomatous angiocentric inflammatory response. Over time, the number of pleomorphic large round cells increases and marked vasoinvasion is observed [11]. The present report describes a case of canine vasotropic and vasoinvasive nonepitheliotropic lymphoma characterized by marked presence of unusual tumor cells with irregularly shaped nuclei and extensive dermal necrosis.

A 14-year-old, spayed female Shih-tzu dog was referred with masses in the dorsal aspect of cervical region and digit of the right anterior limb. The lesions were clinically characterized by ulceration, pyogenic and erosions, accompanied by pruritus. Punch biopsy of the digital mass was performed and cutaneous histiocytoma was diagnosed by a diagnostic laboratory. The masses, however, continued to enlarge and malignant tumors were suspected. The tumor masses were excised surgically under systemic anesthesia 2 weeks after first presentation. At this point, the masses had developed not only in the cervical and digital region, but also in the lower lip and tail. Among the tumor masses excised, cervical and digital masses were submitted to histopathological examination in our laboratory. The cervical mass was 2 cm in diameter, forming a dome-shaped nodular lesion with necrosis of the overlying dermal tissue. On cut section, the mass was grey-white and poorly demarcated. The digital mass was spread throughout the digit, poorly demarcated and with similar features on cut section to those of cervical tumor. No special treatments were subsequently performed. Six months later, recurrence was observed in the cervical and lower lip regions (Fig. 1). Tumor also developed in the pollex of the left anterior limb and in the axilla, and the dog died 1 month after recurrence. Necropsy was not permitted.

The surgically excised tissues were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned and stained using hematoxylin and eosin (HE) and toluidine blue. Immunohistochemical staining was performed using rabbit anti-human CD3 antibody (DAKO, Copenhagen, Denmark), rabbit anti-CD20 antibody (Thermo Fisher Scientific, California, U.S.A.), rabbit anti-human lysozyme antibody (DAKO) and mouse anti-human HLA-DR antigen, alpha-chain antibody (DAKO) as primary antibodies. Peroxidase-conjugated anti-rabbit immunoglobulin (IgG) (Histofine Simple Stain MAX-PO(R); Nichirei, Tokyo, Japan) and peroxidase-conjugated anti-mouse IgG (Histofine Simple Stain MAX-PO(M); Nichirei) was used as a secondary antibodies. Cell proliferating activity and apoptosis of the tumor cells were also examined. Cell proliferating activity was examined immunohistochemically
Histologically, diffuse neoplastic cell proliferation and infiltration were seen from the superficial dermis to the deep dermis, forming a poorly demarcated, solid nodule in the cervical mass (Fig. 2). Two types of neoplastic cells were identified: lymphoblast-like cells with a round-oval, vesicular nuclei and moderate-large nucleoli; and smaller cells with characteristic irregularly shaped dark nuclei (Fig. 3A). Mitotic figures were numerous. Both types of tumor cells were negative for metachromasia on toluidine blue staining and positive for CD3 (Fig. 3B), indicating the T-cell origin of the tumor cells. Ultrastructurally, neoplastic cells with round nuclei were characterized by clumped chromatin in the nuclei, distinct nucleoli and scant cytoplasm, characteristic of lymphoblasts. In contrast, cells with irregularly shaped nuclei presented cerebriform, pleomorphic nuclei and a pattern of chromatin conforming to lymphocytic cells (Fig. 4). These pleomorphic nuclei frequently showed highly condensed chromatin suggesting early events of apoptosis. Immunohistochemically, lymphoblast-like tumor cells commonly expressed high levels of PCNA, whereas expression in cerebriform-nuclei tumor cells was rare and lower in staining intensity. The cerebriform-nuclei tumor cells were frequently positive for cleaved caspase-3 (Fig. 5). However, most of these cleaved caspase-3-positive cells were TUNEL-negative. Most TUNEL-positive cells conformed to the cells with highly condensed nuclei which were confirmed as apoptotic bodies. Most vessel walls had been invaded by tumor cells (Fig. 6), and intravascular infiltration of CD3-positive cells was also identified. In addition, vascular invasion was associated with necrosis of the vascular wall, where histiocytic cells intermingled. Ischemic necrosis and hemorrhage apparently caused by vascular damage by neoplastic cells were present throughout the full thickness of skin, including epidermis, adnexa and dermal tissue. Occasional infiltration of CD3-positive cells was also seen in the epidermis. CD20-positive B cells were seen in tissues surrounding the mass, and focal infiltration of these cells was also seen in the neoplastic mass. In addition, a lysozyme-positive histiocytic cell population was intermingled with neoplastic cells, especially in adipose tissue and around the necrotic vessel walls. These CD20- and lysozyme-positive inflammatory cells were also positive for HLA-DR antigen, whereas CD3-positive neoplastic cells were negative for these antigens. The digital mass displayed severe inflammation, mostly comprising macrophages, although CD3-positive tumor cells intermingled with these inflammatory cells. Among these neoplastic lymphocytes, lymphoblast-like cells with round-oval nuclei dominated.

In humans, vasotropic and vasoinvasive nonepitheliotropic lymphoma is reported as a B-cell proliferation admixed with reactive T-cells, which usually numerically predominate [5, 6]. On the other hand, this case clearly showed CD3-positive T-cell proliferation, which has been reported in other cases of canine vasotropic and vasoinvasive nonepitheliotropic lymphoma [3, 11]. The present study could not examine the particular immunophenotype (CD4, CD8, TCRαβ and TCRγδ) of neoplastic T-cell lymphocytes, as fresh specimens of the tumor were unavailable. No reports have described tumor cell expression for CD4 or CD8 in canine vasotropic and vasoinvasive nonepitheliotropic lymphoma, although CD4-positive cells are reportedly dominant in human cases [10, 11]. As a result, the neoplastic vascular invasion and destruction observed in this case caused ischemic necrosis of neoplastic and non-neoplastic tissues in the skin, representing a characteristic feature of vasotropic and vasoinvasive nonepitheliotropic lymphoma. Inflammatory cells mostly comprising histiocytic cells might be a response of necrotic tissue. Vasotropic and vaso-
invasive nonepitheliotropic lymphoma is also known as ‘lymphomatoid granulomatosis’ [3], which is mainly reported as a pulmonary disease in dogs [1, 2, 7, 8]. As in the human category, lymphomatoid granulomatosis is defined as a disease that does not always progress to neoplastic lymphoid proliferation [4]. As to this case, we considered that ‘vasotropic and vasoinvasive nonepitheliotropic lymphoma’ might be a more suitable diagnosis than ‘lymphomatoid granulomatosis’, because the pathologic appearances of the proliferating lymphocytes were apparently neoplastic and there were no clinical features of pulmonary involvement. The presence of small neoplastic cells with irregularly shaped nuclei was characteristic in this case. These unusual cells ultrastructurally showed characteristics of neoplastic lymphocytes, and proliferative activities of these cells were lower than those of lymphoblast-like tumor cells. Based on these results, we presume that these unusual tumor cells originated from the lymphoblast-like tumor cells, which expressed high levels of cell proliferating activity. Additionally, expression of cleaved caspase-3 and unusual condensed chromatin of the cerebriform-nuclei tumor cells suggested that these unusual neoplastic cells might be in pro-stage of apoptotic reaction cascade. However, if at all this hypothesis was correct, the numerous appearance and long-term sustention of cells in pro-stage of apoptosis seems unusual, so that further examination of the cases resembling present case are required to understand these phenomena.

REFERENCES